Data Evaluation Report on the Toxicity of DPX-MAT28 Technical (Aminocyclopyrachlor) to Rainbow Trout (*Oncorhynchus mykiss*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 47560130

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	358148
	OECD Data Point	{.....}
	EPA MRID	47560130
	EPA Guideline	OPPTS 850.1400

Test material: DPX-MAT28 Technical **Purity:** 92.2%
Common name Aminocyclopyrachlor
Chemical name:
IUPAC: 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS: 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
CAS No.: 858956-08-8
Synonyms: None reported

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature: Christie E. Padova
Date: 07/21/09

Primary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.
Signature:
Date: 07/27/09 Teri S Myers

Secondary Reviewer: Anita Ullagaddi
EPA/OPP/EFED/ERB1
Signature
Date: 10/06/09

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	None	

Date Evaluation Completed: 10/06/09

CITATION: Gallagher, S.P., T.Z. Kendall, and H.O Krueger. 2008. DPX-MAT28 Technical: An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Study No. 112A-239. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, DE. Study initiated January 22, 2008 and submitted September 3, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 90-day chronic toxicity of DPX-MAT28 Technical (aminocyclopyrachlor) to the early life-stage of rainbow trout (*Oncorhynchus mykiss*) was studied under flow-through conditions. Fertilized eggs/embryos (120/level, <24 hours old) of rainbow trout were exposed to nominal concentrations of 0 (negative control), 0.75, 1.5, 3.0, 6.0, and 12 mg ai/L. TWA concentrations were identical to mean-measured concentrations and were <0.00245 (<LOD, control), 0.69, 1.5, 2.9, 5.8, and 11 mg ai/L, respectively. The test system was maintained at 12 ± 1°C and a pH of 8.0 to 8.3. There were no treatment-related effects on hatching success, time to hatch, post-hatch survival, time to swim-up, or growth at any treatment level. The 90-day LC/EC_{50} for all endpoints was >11 mg ai/L, and the NOAEC and LOAEC were 11 and >11 mg ai/L, respectively.

This study is scientifically sound and classified as acceptable. It satisfies guideline requirements for an early life stage toxicity study with fish.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Embryos, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

LOAEC: >11 mg ai/L
NOAEC: 11 mg ai/L

Endpoints affected: none

I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED: U.S. EPA OPPTS No. 850.1400 (1996)
OECD Guideline No. 210 (1992)
U.S. EPA SEP 540/9-86-138 (1986)

There were no guideline deviations affecting the scientific soundness or acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the GLP Standards as published in the 40 CFR, Part 160 with the following exception: periodic analysis of well water for potential contaminants.

A. MATERIALS:

1. Test Material DPX-MAT28 Technical (aminocyclopyrachlor)

Description: Solid

Lot No./Batch No.: 009

Purity: 92.2%

Stability of compound under test conditions: Verified: concentrations of DPX-MAT28 Technical (aminocyclopyrachlor) were adequately maintained within 20% of the TWA concentrations for each level.

Storage conditions of test chemicals: Ambient temperature

Physicochemical properties of Aminocyclopyrachlor.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)
[*EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow,*

and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.]

Age /embryonic stage at test initiation: Embryos, <24 hours post-fertilization.
[*EPA recommends fish embryos 2 to 24 hours old.*]

Method of collection of the fertilized eggs: Gametes from at least three females and three males were received and fertilized at Wildlife International, Ltd.

Source: Unfertilized eggs and sperm were obtained from Troutlodge, Inc., Sumner, WA.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The concentrations were selected in consultation with the Sponsor and were based upon the results of exploratory range-finding toxicity tests (not further described).

c. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions (same as test or not): Feeding (type, source, amount given, frequency): Health: (any mortality observed)	N/A	
Number of fertilized eggs/embryos in each treatment at test initiation	120 embryos per treatment level, divided into 15 embryos per cup, two cups per replicate, and four replicate chambers per level.	On day 17 post-hatch, when >90% of the control larvae reached swim-up, the number of larvae in all replicates was reduced to 15 (60 per level) to prevent overcrowding. *Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)*

Parameter	Details	Remarks *Criteria*
Concentration of test material nominal: mean measured and TWA (reviewer-calculated): :	0 (negative control), 0.75, 1.5, 3.0, 6.0, and 12 mg ai/L <0.00245 (<LOD, control), 0.69, 1.5, 2.9, 5.8, and 11 mg ai/L	For concentration verification, samples were analyzed from two alternating replicate chambers at test initiation, at approximately weekly intervals during the test, and at test termination. Water samples were analyzed by direct-injection using an HPLC equipped with UV (220 nm) detection. TWA concentrations were reviewer-calculated using Excel software (copy of worksheet in Appendix II), and results were essentially identical to the study author's mean-measured concentrations (using two significant figures). The majority of analytical results were within 20% of mean-measured concentrations. *A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured in one tank at each toxicant level every week.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage.* *OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within ± 20% of the mean measured values.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	*The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol. OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L.*
Number of replicates control: solvent control: treated ones:	 4 N/A 4/level	*Number of replicates should be 4 per concentration. A solvent control should be used in conjunction with a solubilizing agent.*
Test condition static renewal/flow-through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 Flow-through Continuous-flow diluter Approximately 6 volume exchanges per day. N/A	Diluter operation was checked twice daily. The flow rates varied by ≤10% of the mean for the four replicates. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used. Toxicant Mixing: 1) Mixing chamber is preferred; 2) Aeration should not be used for mixing; 3) The test solution should be completely mixed before introduction into the test system; 4) Flow splitting accuracy should be within 10%.*

Parameter	Details	Remarks *Criteria*
Aeration, if any	Gentle aeration was initiated on Day 70 to accommodate increased oxygen demands of the growing fish.	*Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	90 days: 25-day hatching period and 65-day post-hatch period	Acceptable for this species under OPPTS guidance. *Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.*
Embryo cups, if used type/material (glass/stainless steel): size: fill volume:	Glass cylinders with 425-µm nylon screen mesh bottoms attached with silicone adhesive 50-mm diameter Not reported	Embryo cups (two) were suspended in each replicate vessel, and were oscillated at 2 rpm. *Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel type/material: (glass/stainless steel) size: fill volume:	Glass 9 L 7 L (15.5-cm depth)	*Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water	Moderately-hard freshwater was obtained from a 40-m deep well located at the laboratory. The well water was sand-filtered, aerated, filtered again (0.45-µm), and UV-sterilized prior to use. The following parameters were measured weekly for 4 weeks preceding the test: specific conductance 295 to 310 µmhos/cm, hardness 136 to 140 mg/L as $CaCO_3$, alkalinity 178 to 180 mg/L as $CaCO_3$, and pH 8.1 to 8.2.	*Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

Parameter	Details	Remarks *Criteria*
Water parameters hardness: pH: dissolved oxygen: temperature (s) (record all the temperatures used for different life stages): photoperiod: salinity (for marine or estuarine species): other measurements: interval of water quality measurements:	 132-144 mg/L as $CaCO_3$ 8.0 to 8.3 ≥7.2 mg/L (≥66% saturation) 11.6-12.8°C (all stages) Embryos and larvae were maintained in relative darkness until 1 week post-hatch. Thereafter, subdued lighting was maintained on a 16-hr light:8-hr dark photoperiod (with 30-minute transition periods). N/A Conductivity ranged from 260 to 275 µmhos/cm; and total alkalinity ranged from 174 to 180 mg/L as $CaCO_3$. Temperature and pH were measured in all (temp.) or alternating (pH) replicates of all levels at the beginning and end of the study, and at weekly intervals during the study. Temperature was also measured continuously in one negative control replicate. DO was measured in alternating replicates of all levels at the beginning of the study, daily for the first 7 days of the study, at least weekly thereafter, and at study termination. Hardness, conductivity, and alkalinity were measured in alternating replicates of the negative control and 12 mg ai/L (high) levels at test initiation and termination, and at weekly intervals during the study.	Hardness and pH range requirements are not reported in OPPTS guidance. A light intensity of 79 lux was measured over one representative chamber on the day the lighting regimen began (1 week post-hatch). *Recommended hardness: 40-48 mg/L as $CaCO_3$;* *Recommended pH:* 7.2 to 7.6 *Dissolved Oxygen (DO) should be measured at each concentration at least once a week;* *Freshwater parameters in a control and one concentration should be analyzed once a week.* *Temperature depends upon test species and should not deviate by more than 2°C from appropriate temperature.* *OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*

Parameter	Details	Remarks *Criteria*
Post-hatch details when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: on what day, the alevins were released from the incubation cups to the test chamber:	Day 25, when hatching in the negative control reached >80% All alevins were released; however, on post-hatch day 17, the number of larvae in all replicates was reduced to 15 to prevent overcrowding. Day 25	Percent hatch ranged from 93 to 97% (mean of 95%) in the four negative control cups. *Percentage of embryos that produce live fry should be ≥ 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.*
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	Day 42 (end of swim-up stage) Salmon-starter mash (Zeigler Brothers, Inc., Gardners, PA) two to three times daily. Rations were adjusted weekly to account for mortality.	Uneaten food and/or debris were removed as needed. The fish were not fed *ca.* 51 hours prior to test termination.
Stability of chemical in the test system	Stable, as indicated by weekly analysis of the test water from all treatment levels. Results were largely within 20% of the mean-measured concentrations.	Fulfills OPPTS criteria.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	99.5 ± 2.65 (CV = 2.66%) At least once weekly 0.00245 mg ai/L 0.500 mg ai/L	Based on QC samples fortified and analyzed with each sample set.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	Mean viability = 98% 30 embryos/cup, with four replicate incubation cups Day 12	
Other parameters, if any	Biomass loading at the end of the test was 0.38 g fish/L/day. Instantaneous loading was 3.9 g fish/L at any given time.	Fulfills OPPTS criteria.

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Hatching success - Time to hatch - Time to swim-up - Post-hatch survival - Post-hatch growth - Clinical signs of toxicity	*Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and Juveniles:* *- Time to swim-up (if appropriate);* *- Measurement of growth;* *- Incidence of pathological or Histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.,alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	 Daily Daily Daily Daily Days 55 and 90 Daily Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No treatment-related effects on hatching success or post-hatch survival were observed. Hatching success averaged 91 to 96% for all levels, with no statistically-significant differences from the negative control observed. Post-hatch survival was assessed at two time periods: from the beginning of the post-hatch period to thinning on Day 17 post-hatch, and from thinning to test termination. Survival from post-hatch to thinning as well as from thinning to test termination was 98 to 100% for all levels, with no statistically-significant differences from the negative control observed. The NOAEC for all survival endpoints was 11 mg ai/L, based on mean-measured concentrations.

Table 3: Effect of DPX-MAT28 Technical (Aminocyclopyrachlor) on Egg Hatching and Survival at Different Life Stages of Fish.

Treatment mg ai/L Mean-measured (and Nominal) Concentrations[a]	No. of eggs at study initiation	At Hatch		At Thinning[b]		At Termination	
		No.	%	No.	%	No.	%
Negative control	120	114	95	112	98	59	98
0.69 (0.75)	120	115	96	114	99	60	100
1.5 (1.5)	120	109	91	108	99	60	100
2.9 (3.0)	120	109	92	108	99	60	100
5.8 (6.0)	120	111	93	110	99	59	98
11 (12)	120	112	93	112	100	61[c]	100
NOAEC[d]		11 mg ai/L		11 mg ai/L		11 mg ai/L	
EC_{50} (with 95% C.I.)[d]		>11 mg ai/L		>11 mg ai/L		>11 mg ai/L	
Positive control mortality: EC_{50}: NOAEC	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] Reviewer-calculated TWA concentrations were identical to the mean-measured concentrations.
[b] Fish were thinned to 60 per level on Day 42 (17 days post-hatch).
[c] Replicate C was inadvertently thinned to 61 fry.
[d] Study author's toxicity values were based on the mean-measured concentrations

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Times to hatch and swim up: No treatment-related effects on the time to hatch or time to swim-up were observed. Embryos began hatching on Day 23 and all surviving embryos from all levels had hatched by Day 26. Trout larvae began swimming up from the bottom of the chambers 11 days post-hatch, and by 17 days post-hatch, 94% of the control larvae had attained swim-up, compared to 91,91, 96,95, and 97% for the 0.69, 1.5, 2.9, 5.8, and 11 mg ai/L levels, respectively. The NOAEC for these endpoints was 11 mg ai/L.

Clinical signs of toxicity: No treatment-related clinical signs of toxicity were indicated during the study. One instance of a curled spine was observed from 1 to 3 weeks post-hatch at the 5.8 mg ai/L level. Another fish from the control group was swimming erratically at the end of week 3 post-hatch. The NOAEC for signs of toxicity was 11 mg ai/L.

Growth: The mean total length was assessed at 30 and 65 days post-hatch, and the mean wet weight and dry weight was assessed at 65 days post-hatch (study termination). No treatment-related effects were observed, with no statistically-significant differences from the control group indicated. At 30 and 65 days post-hatch, mean total lengths ranged from 34.8 to 35.3 mm and from 57.2 to 58.1 mm for all levels, respectively. At 65 days post-hatch, mean wet weights and dry weights ranged from 1.84 to 1.91 g and from 0.39 to 0.41 g, respectively. The NOAEC for all growth parameters was 11 mg ai/L.

Table 4: Effect of DPX-MAT28 Technical (Aminocyclopyrachlor) on Growth of Juvenile Fish.

Treatment mg ai/L Mean-measured (and Nominal) Concentrations[a]	First day to hatch	% Swim-up (day 17 post-hatch)	Growth			
			30 days post-hatch	65 days post-hatch		
			Mean total length (mm ± SD)	Mean total length (mm ± SD)	Mean wet weight (g ± SD)	Mean dry weight (g ± SD)
Negative control	24.0	94	35.3 ± 0.45	57.5 ± 0.93	1.84 ± 0.044	0.39 ± 0.010
0.69 (0.75)	24.0	91	35.1 ± 0.14	57.2 ± 0.41	1.87 ± 0.024	0.39 ± 0.008
1.5 (1.5)	23.5	91	35.0 ± 0.50	58.0 ± 0.50	1.91 ± 0.026	0.41 ± 0.006
2.9 (3.0)	23.5	96	34.8 ± 0.13	57.5 ± 1.06	1.85 ± 0.061	0.39 ± 0.014
5.8 (6.0)	23.5	95	35.1 ± 0.36	57.8 ± 0.40	1.88 ± 0.049	0.40 ± 0.011
11 (12)	24.0	97	35.0 ± 0.12	58.1 ± 0.85	1.91 ± 0.022	0.41 ± 0.005
NOAEC [b]	11 mg ai/L	11 mg ai/L	11 mg ai/L			
EC_{50} [b]	>11 mg ai/L	>11 mg ai/L	>11 mg ai/L			
Positive control mortality: EC_{50}: NOAEC	N/A	N/A	N/A			

[a] Reviewer-calculated TWA concentrations were identical to the mean-measured concentrations.
[b] Study author's toxicity values were based on the mean-measured concentrations.

C. REPORTED STATISTICS:

Endpoints evaluated by statistical analysis included hatching success, time for larvae to swim-up, percent survival of larvae prior to and following swim-up, fish total length on day 30 post-hatch and at test termination, and wet and dry weight of fish at test termination. Time to swim-up was evaluated by calculating the number of larvae that had achieved swim-up relative to the number of surviving larvae on any one day. Effects on time to swim-up were evaluated by a comparison of the numbers of larvae in each treatment group that had achieved swim-up by day 17 post-hatch (completion of swim-up). Post-hatch survival from hatching to swim-up was calculated as the number of larvae alive prior to thinning on day 17 post-hatch relative to the total number of larvae that had successfully hatched. Post-hatch survival from thinning to study termination was calculated as the number of juvenile fish alive on day 65 post-hatch relative to the number of larvae remaining after thinning.

Continuous-variable data (i.e., growth data) were evaluated for normality using the Chi-square test and for homogeneity of variance using Levene's test ($p=0.01$). All growth data passed both assumptions and were evaluated with ANOVA and Dunnett's t-test ($p=0.05$). Discrete-variable data (hatching success, time to swim-up, and survival) were analyzed using Chi-square and Fischer's Exact test to identify treatment groups that showed a statistically-significant difference ($p\leq0.05$) from the control. The LOAEC, NOAEC, and MATC were reported based upon significance of the data. All statistical tests were performed using mean-measured concentrations and SAS or TOXSTAT statistical software.

The LC/EC_{50} values were estimated to be greater than the highest treatment level.

NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L
MATC: >11 mg ai/L
Endpoints affected: none

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer statistically-verified the results for hatching success only using Toxstat statistical software. Data were determined to be normally distributed (using the Chi-square and Shapiro-Wilks tests) and the variances were homogeneous (using the Hartley and Bartlett's tests); the NOAEC was determined using ANOVA, followed by Dunnett's test. All other endpoint results could be visually verified due to promotion or no change in the treated groups, relative to the negative control group. It was clearly determined through visual inspection of the data that there were no effects of treatment on any endpoint in this study.

NOAEC: 11 mg ai/L
LOAEC: >11 mg ai/L
MATC: >11 mg ai/L
Endpoints affected: none

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study authors'.

TWA concentrations were reviewer-calculated (refer to associated Excel worksheet in Appendix II) and were essentially identical to the mean-measured concentrations (assuming two significant figures) reported by the study author. TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C_{TWA} is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =96 hours)

During the morning of Day 8, particulate matter was observed in the stock delivery line for the nominal 12 mg ai/L level, which appeared to decrease the flow of the stock solution. The particulate matter was removed and the flow completely restored. Due to this particulate formation, the stock solution concentration was decreased and the stock flow rate was increased on Day 8. During the morning of Day 43, black particulate matter was observed in the stock delivery line for the nominal 12 mg ai/L level. The particulate matter was removed, and no other particulate matter formation was noted in the system during the test.

Stock solutions were prepared 29 times during the study. For the first three preparations (used during the pre-test period and first 8 days of the definitive study), a 6-L primary stock was prepared in well water at 3.0 mg ai/L. The stock was prepared using sonication (40 to 45 minutes) and mixed by inversion, and appeared clear and colorless. Proportional dilutions were then made from this primary stock using additional well water. Beginning on Day 8, the primary stock was changed to 1.5 mg ai/mL in order to reduce the potential for particulate formation in the delivery lines.

Stock and dilution water flows were increased on Day 62 in an attempt to accommodate the increased DO demand of the growing fish.

Measured values of aminocyclopyrachlor were within 20% of TWA concentrations with the exception of occasional short-term decreases in recoveries due to equipment malfunctions. On Day 7, recoveries of approximately 50% of nominal were observed at the nominal 12 mg ai/L level due to precipitate formation in the delivery lines. The precipitate was removed and recoveries of *ca.* 70% were revealed the morning of Day 8. Although precipitate formation was again observed in the delivery lines at this level on the morning of Day 43, recoveries of *ca.* 83% on Day 42 indicated that the stock flows had been minimally impacted and for a relatively brief period of time. On Days 60 and 64, initial recoveries of *ca.* 50% of nominal were measured at the nominal 0.75 mg ai/L level; additional samples analyzed on these days resulted in recoveries of *ca.* 90% of nominal; it was reported that the low sample results were not included in the mean-measured concentration due to a temporary equipment malfunction.

The in-life phase of the definitive study was conducted from January 24 to April 23, 2008.

G. CONCLUSIONS:

This study is scientifically sound and classified as acceptable. There were no treatment-related effects on hatching success, time to hatch, post-hatch survival, time to swim-up, or growth of rainbow trout (*Oncorhynchus mykiss*) during a 90-day early life stage toxicity test conducted at up to 11 mg ai/L.

LOAEC: >11 mg ai/L
NOAEC: 11 mg ai/L

Endpoints affected: none

III. REFERENCES:

Organization for Economic Cooperation and Development. 1992. OECD Guideline for Testing of Chemicals, 210 *Fish, Early-Life Stage Toxicity Test.*

U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1400: *Fish Early Life-Stage Toxicity Test.*

U.S. Environmental Protection Agency. 1986. *Standard Evaluation Procedure, Fish Early Life-Stage Test.* Office of Pesticide Programs, Hazard Evaluation Division. EPA 540/9-86-138.

APHA, AWWA, WPCF. 1995. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation. New York.

Martin, J.W. 1967. *A method of measuring lengths of juvenile salmon from photographs.* Progr. Fish-Cult. 29:238-240.

West, Inc., and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, WY.

The SAS System for Windows. 2001. Version 8.2. SAS Institute Inc., Cary, North Carolina.

APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
hatching success
File: 0130h       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.608         5.808             9.168           5.808       1.608
OBSERVED     0             9                 5              10           0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   9.8908
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


hatching success
File: 0130h       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =   26.000

W =    0.957

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


hatching success
File: 0130h       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =    8.75
Closest, conservative, Table H statistic =  184.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       3
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =   3.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
```

but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

hatching success
File: 0130h Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.48
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

hatching success
File: 0130h Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7.833	1.567	1.085
Within (Error)	18	26.000	1.444	
Total	23	33.833		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

hatching success
File: 0130h Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	28.500	28.500		
2	0.69	28.750	28.750	-0.294	
3	1.5	27.250	27.250	1.471	

4	2.9	27.250	27.250	1.471
5	5.8	27.750	27.750	0.883
6	11	28.000	28.000	0.588

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

hatching success
File: 0130h Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.69	4	2.048	7.2	-0.250
3	1.5	4	2.048	7.2	1.250
4	2.9	4	2.048	7.2	1.250
5	5.8	4	2.048	7.2	0.750
6	11	4	2.048	7.2	0.500

hatching success
File: 0130h Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	28.500	28.500	28.625
2	0.69	4	28.750	28.750	28.625
3	1.5	4	27.250	27.250	27.563
4	2.9	4	27.250	27.250	27.563
5	5.8	4	27.750	27.750	27.563
6	11	4	28.000	28.000	27.563

hatching success
File: 0130h Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	28.625				
0.69	28.625	0.147		1.73	k= 1, v=18
1.5	27.563	1.103		1.82	k= 2, v=18
2.9	27.563	1.103		1.85	k= 3, v=18
5.8	27.563	1.103		1.86	k= 4, v=18

```
                  11     27.563     1.103              1.87       k= 5, v=18
-------------------------------------------------------------------------------
s =    1.202
Note: df used for table values are approximate when v > 20.
```

APPENDIX 2: COPY OF REVIEWER'S TWA CALCULATIONS:

Nominal Concentration (mg ai/L)	Time (Day)	Measured Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	TWA (mg ai/L)
0.75	0	0.71		
	0	0.716	0.713	
	7	0.701		
	7	0.704	0.7025	
	14	0.665		
	14	0.666	0.6655	
	21	0.714		
	21	0.716	0.715	
	28	0.724		
	28	0.726	0.725	
	35	0.637		
	35	0.642	0.6395	
	42	0.698		
	42	0.704	0.701	
	49	0.682		
	49	0.687	0.6845	
	56	0.692		
	56	0.695	0.6935	
	60			
	60	0.679	0.679	
	62	0.679		
	62	0.68	0.6795	
	64	0.668		
	64	0.669	0.6685	
	70	0.686		
	70	0.691	0.6885	
	77	0.726		
	77	0.731	0.7285	
	84	0.721		
	84	0.722	0.7215	
	90	0.666		
	90	0.671	0.6685	
				0.6942306
1.5	0	1.63		
	0	1.64	1.635	
	7	1.48		
	7	1.48	1.48	
	14	1.42		
	14	1.42	1.42	
	21	1.45		
	21	1.46	1.455	

	28	1.38		
	28	1.39	1.385	
	35	1.42		
	35	1.43	1.425	
	42	1.44		
	42	1.45	1.445	
	49	1.42		
	49	1.42	1.42	
	56	1.52		
	56	1.53	1.525	
	62	1.45		
	62	1.46	1.455	
	70	1.42		
	70	1.42	1.42	
	77	1.45		
	77	1.45	1.45	
	84	1.44		
	84	1.44	1.44	
	90	1.39		
	90	1.4	1.395	
				1.4486111
3	0	2.75		
	0	2.76	2.755	
	7	2.81		
	7	2.82	2.815	
	14	2.79		
	14	2.79	2.79	
	21	3.06		
	21	3.08	3.07	
	28	2.95		
	28	2.96	2.955	
	35	2.76		
	35	2.77	2.765	
	42	2.88		
	42	2.9	2.89	
	49	2.98		
	49	3	2.99	
	56	2.95		
	56	2.96	2.955	
	62	2.87		
	62	2.88	2.875	
	70	2.91		
	70	2.92	2.915	
	77	2.87		
	77	2.89	2.88	
	84	2.84		

	84	2.85	2.845	
	90	2.81		
	90	2.81	2.81	
				2.8871667
6	0	5.88		
	0	5.91	5.895	
	7	5.96		
	7	5.97	5.965	
	14	5.77		
	14	5.79	5.78	
	21	5.72		
	21	5.84	5.78	
	28	5.48		
	28	5.58	5.53	
	35	5.55		
	35	5.53	5.54	
	42	5.98		
	42	6.04	6.01	
	49	6.06		
	49	6.08	6.07	
	56	6.02		
	56	6.04	6.03	
	62	5.68		
	62	5.79	5.735	
	70	5.85		
	70	5.86	5.855	
	77	5.75		
	77	5.87	5.81	
	84	5.98		
	84	6.38	6.18	
	90	5.6		
	90	5.62	5.61	
				5.8475556
12	0	11.6		
	0	11.8	11.7	
	7	**6.14**		
	7	**5.97**	6.055	
	8	**8.61**		
	8	**8.43**	8.52	
	14	10.6		
	14	10.7	10.65	
	21	11.9		
	21	12	11.95	
	28	10.8		
	28	10.9	10.85	

35	11.2	
35	11.3	11.25
42	9.99	
42	10.1	10.045
49	11.3	
49	11.3	11.3
56	11.6	
56	11.7	11.65
62	12.1	
62	12.2	11.75
70	11.3	
70	11.3	11.2
77	11.1	
77	11.3	11.7
84	12.1	
84	12.2	11.7
90	11.2	
90	11.2	11.2

10.950056

Bolded values are outside the ±20% TWA range